Methyl bromide alternatives

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USDA

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Inside This Issue

Update on Montreal Protocol Actions

"Methyl bromide is a tough subject because everyone involved with the issue needs some certainty for the future," said Charlie Rawls. Speaking at the 1997 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions in San Diego, California, Nov. 3–5, he was updating the actions taken by the Montreal Protocol in 1997. Rawls is executive assistant to Richard Rominger, deputy secretary of the U.S. Department of Agriculture.

According to Rawls, the Montreal Protocol will review an updated version of the Methyl Bromide International Assessment document in 1999 to determine what additional actions should be taken.

"There is likely to be a push to move up the phaseout dates for developed and developing countries. However the discussions should primarily focus on the phaseout date for developing countries, and dates for freezing production and consumption levels and further interim reductions."

Rawls compared the U.S. Clean Air Act with the Montreal Protocol (MP).

"The Clean Air Act classifies ozone-depleting substances as Class I and II materials and mandates their phaseout, while the Montreal Protocol makes no classification, leaving phaseouts to be voted on by member countries. And while the Clean Air Act provides for no exemptions or essential uses, the Montreal Protocol allows both."

Two exemptions were approved by the 1997 meeting of the Parties to the Montreal Protocol:

- An emergency-use provision, which will allow a country to use up to 20 tons of methyl bromide in emergency situations such as unanticipated pest outbreaks or infestations,
- A "critical use" exemption to allow use of methyl bromide where no available alternatives exist after the phaseout date.

Criteria for critical use, Rawls said, would include situations "where significant market disruptions would occur without the use

This newsletter provides information on research for methyl bromide alternatives from USDA, universities, and industry.

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and where no technically and economically feasible alternative is available. Production of methyl bromide would be permitted for critical uses only if there were no existing stocks and if there were no feasible alternatives available. However, there must be evidence that appropriate measures were being taken to identify alternatives.

In 1992, the Montreal Protocol agreed to freeze production and use of methyl bromide at the 1991 levels in developed countries on January 1, 1995, and at the 1995–98 average in developing countries on January 1, 2002. Actions taken at the September 9–17, 1997, meeting in Canada are shown in the table below.

Rawls reported that USDA's Economic Research Service (ERS) will lead a study of the impact the ban will have on the U.S. agricultural economy. Scientists from ARS' National Agricultural Pesticide Impact Assessment Program and from the National Center for Food and Agricultural Policy will work with ERS to establish baselines for uses of methyl bromide and review available alternatives for commodities and growing areas of the country. They will study changes in crop yields and costs of production associated with farming without methyl bromide or viable alternatives.

"These USDA agencies will work with EPA and with industry and growers, possibly through workshops or forums, to gather data and review the process of economic analysis," Rawls said. "Through cooperative agreements, we hope to calculate the economic impact of the methyl bromide phaseout on producers, consumers, and our trade situation. The first phase of the study is well under way and the full report is expected by June or July 1998."

Since there is such divergence between the mandates of the U.S. Clean Air Act and the Montreal Protocol, legislation is needed to address these differences. Rawls said that "a reasonable approach would be to consider amending the Clean Air Act to ensure that regulations governing use, production, import, or export of methyl bromide in the United States be no more stringent or restrictive than those required by the Montreal Protocol. This would bring the United States in line with other developed countries and provide certainty for those with an interest in the production or use of methyl bromide."

Action	Developed Countries	Developing Countries
Interim cuts		
1999	25 percent	0 percent
2001	50 percent	0 percent
2003	70 percent	review
2005	—	20 percent
Phaseout	2005	2015
Exemptions	Quarantine and preshipment	Quarantine and preshipment
	Critical Use* Emergency*	Critical Use* Emergency*

* Effective only after the phaseout.

Excerpt of Remarks by David R. Riggs, Chairman of the Crop Protection Coalition 1997 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, San Diego, California, November 3–5

In the methyl bromide issue, we've arrived at the point where "the rubber meets the road!" The time for real, workable solutions is now.

At the 1997 Montreal Protocol meeting, industrialized nations of the world agreed to phase out methyl bromide in 2005, with interim reductions of 25 percent in 1999, 50 percent in 2001, and 70 percent in 2003. The interim 70-percent reduction in 2003 is a de facto ban. Meanwhile, the developing nations have committed only to freezing the use of methyl bromide at their average use between 1995 and 1998. They'll reduce use from that level by 20 percent in 2005 and phase out use in 2015.

So our major competitors have virtually unlimited access to methyl bromide well into the next century. This regulatory inconsistency makes the economic viability of alternatives more questionable. What may be economically viable if everyone loses methyl bromide may not be viable if only some producers lose it.

American farmers are still facing the complete loss of methyl bromide in 2001 under the Clean Air Act. Under terms of the Montreal Protocol treaty, we would also be required to reduce the use of methyl bromide 25 percent in 1999. Even if there is some legislative adjustment to the Clean Air Act, we don't know just how far Congress may go in resolving this inequity. Regardless, time is very short.

The Crop Protection Coalition has been very aggressive in supporting increased funding for methyl bromide alternatives research, and we've argued that funding should go to USDA–ARS and to ongoing projects at universities. We envisioned a close partnership among ARS scientists, university researchers, and farmers to deliver results to meet users' needs. Although we don't think this partnership has developed to the point necessary to successfully address the urgent situation we face, we hope this conference will help launch us in that direction.

Obviously, the phaseout schedule we face adds greater urgency to your work. We need real solutions in the short term and improved solutions over time. But we must accept that time is short: 1999, 2001, 2003 are **real** dates and they're **real** close. These dates bring real consequences for farmers, farm workers, and communities if we're not successful.

We would like to make the following recommendations for researchers:

- Put a high priority on reaching out to the industries involved in your research.

- Get to know farmers and trade groups and develop relationships relevant to crops being researched.

- Get to know economic, environmental, and regulatory issues that must be considered for growers of those crops to remain viable. When considering an alternative, think of how it will work and what will be necessary to make it work.

- Frame research and findings in the context of field situations. Even the most basic research should have field-level relevance, and highly theoretical research can yield useful, short-term, practical knowledge, data, and recommendations.

- Develop a matrix to identify crops that rely on methyl bromide; pathogens to be controlled; and suggested or likely alternatives with their development stage, advantages, and disadvantages, known economic and production consequences, and implementation restraints. Such a matrix would better define priorities and identify research most likely to end up as technology that farmers can use.

It is clear that special-use exemptions may be a part of the regulatory future of methyl bromide. Realistic data on production and efficiency loss, ancillary impacts, and real tradeoffs of potential alternatives will all be very important in any special-use exemption process. Research will be vital in that process.

As scientists, farmers of crops you are researching will challenge you for results they can act on in the short term. They'll press you to collaborate with other researchers in other institutions and disciplines. Farmers not yet involved in research on alternatives may come to you in July 2000 and ask what you're going to do to save them over the next 6 months. Again, urgency is the message of the day. As you look at your research agenda, ask yourself, "By the time I develop a solution, will the farmer still be around to use it?"

A few thoughts on perspective: We expect technology that we can readily implement from your research. Our goal is not to change the face of farming. The Crop Protection Coalition has repeatedly asserted that the most likely alternatives to methyl bromide are those which evolve farming practices rather than those which call for radical changes.

It was during the Civil War that Abraham Lincoln founded the USDA and the nation's Land Grant University System—both to educate citizens and to help American farmers solve farming problems through research and education. The result of Lincoln's foresight is an American agriculture that is the envy of the world.

The impending loss of methyl bromide is yet another important test of this system. It presents not only a great challenge, but also a great opportunity to show the power of agricultural research. We in the Crop Protection Coalition look forward to doing our part.

Florida Strawberry Growers' Perspective on the Methyl Bromide Issue

"When it comes to methyl bromide, one fact is a given: every situation is unique," said Charles (Chip) Hinton. "As each commodity reacts differently to treatment with methyl bromide, each grower group reacts differently to the loss of this treatment. Likewise, solutions to the problem of the loss of methyl bromide are not universal."

Hinton was reporting the Florida strawberry growers' perspective on the January 1, 2001, ban on methyl bromide. He made these remarks at the Annual International Research Conference on Methyl Bromide Alternatives and Emissions held in San Diego, November 3–5, 1997.

"When growers began using methyl bromide in 1964, we saw an immediate fourfold increase in productivity because of industry-wide acceptance of what was touted as a risk-free, broad-spectrum pest control," he said.

According to Hinton, there is extreme pest pressure in the sandy soils of Florida that have less than 1 percent organic matter. "We still have small family farms here averaging about 35 acres all surrounded by suburbia, that practice an unforgiving planting regime such as double cropping. Because of our climate, we farm in the wintertime, just the opposite of the rest of the country."

Florida growers, he said, can be hit with flooding caused by 20 inches of rain from one storm, as well as high winds, drought, and other weather extremes that greatly affect crops. These extremes rule out the feasibility of using some suggested alternatives to methyl bromide.

Chemical Alternatives

Researchers in Florida have experimented with several potential chemical alternatives to methyl bromide. These include vapam, basamid, chloropicrin, and Telone C17 and C35. But, he said, these all have limitations because of the sandy soils. The chemicals could leach down into the water table because the liquids won't move horizontally. We need horizontal movement to control weeds on bed shoulders.

Some products would be costly, labor intensive, and possibly corrosive, while their efficacy could be inconsistent. Many chemicals emit noxious odors, which would make them difficult to use in our farming communities that border suburbia.

The safety of workers and their families is vital when considering a chemical alternative to methyl bromide. Since the small growers have only a few acres, they're working in their own backyards. They and their families will be exposed to anything applied to their fields.

And finally, some chemicals may present more of a threat to the environment than methyl bromide. There just hasn't been enough research done on the chemical alter-

natives. Some of the proposed alternatives are chemicals discarded 30 years ago because they were not effective. Can these same chemicals meet the standard of being an alternative today?

Nonchemical Alternatives

Hinton talked of nonchemical alternatives such as cover crops, heat treatments, steam solarization, vertical culture, and growing in tunnels. He said that Florida growers have used hairy indigo, peas, mustard, and marigolds as cover crops to attract more beneficial insects and require less pesticides. Although the peas added additional nitrogen to the soil, they did nothing for the nematodes. It was difficult for growers to get a good stand of indigo, mustard, or marigold crops. Results at best, he said, "were spotty."

"We've also tried integrated pest management, flooding, nomadic agriculture, artificial substrates, and adding organic amendments to our soil," he said. Some of these practices just weren't practical. Water is metered for Florida growers, so flooding isn't possible. It's hard to practice nomadic agriculture, or rotating crops, on 35 acres or less. And artificial substrates may work in the greenhouse, but Florida's climate comes into play here. Organic amendments would help, but supply and distribution are problems. Where would growers get the amendments? Would it be cost-effective to transport the material long distance?

The bottom line, he said, is that Florida, which supplies the United States with winter vegetables, is faced with problems like nutsedge, sting nematode, new pests, and soilborne diseases—with no good substitute to methyl bromide on the horizon.

There is now a new group of weeds that in the past was not an economic problem. Some fields have now been without methyl bromide for 3 years. Some of these are fields that have been grown in strawberries for the past 100 years. So there are new pest problems developing. Florida historically hasn't been plagued with soilborne diseases, but without methyl bromide, these can become economically devastating.

"We haven't had to worry about below-ground, arthropod pests. But, we anticipate that they will also become a worry.

"And the alternatives that researchers have come up with so far all cost more and produce less than methyl bromide," Hinton said. "We have cooperated with ARS, CSREES, and the Land Grant institutions in trying to solve this problem of finding alternatives we can use. We've received funds from the Federal Government. At the State level, researchers from the University of Florida have worked hard to develop and test potential alternatives. But, so far, we only have partial solutions to this very serious problem.

"The strawberry growers of Florida understand the complexity of long-term research and realize that time is short before methyl bromide is banned in the United States. We face a real dilemma if viable alternatives aren't found soon or the time for finding them isn't extended."

Soil Bacterium Reduces Methyl Bromide Emissions

Scientists with the U.S. Geological Survey (USGS) have found a type of bacterium in the soil that literally eats methyl bromide and thrives on it.

Ronald S. Oremland leads this research for USGS in Menlo Park, California. His work was reported by colleague Tracy L. Connell at the San Diego 1997 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, November 3–5, 1997.

"The possibility that soil bacteria directly oxidize methyl bromide, thereby contributing to its destruction, is generally overlooked," said Oremland, a USGS geochemist. "This is because both methyl bromide and chloropicrin, which is often used with methyl bromide, are potent biocides that are presumed to kill off, or inactivate, any soil bacteria as well as target organisms."

In lab experiments, Oremland and colleagues isolated a pure culture of a gram negative, motile rod-shaped bacterium from soils that had been fumigated with methyl bromide. They designated the bacterium as strain IMB–1.

This strain of bacterium is closely related to *Rhizobium*, the species of common bacteria that converts

nitrogen to a form useful in the soil.

"We found that this new strain not only thrives on methyl bromide, but it can also grow by oxidizing methyl iodide, methyl chloride, methylated amines, acetate, and glucose," Oremland said. "In lab tests when grown on substrates of all of these compounds, IMB–1 retained its ability to break down methyl bromide, even after being carried through two consecutive transfers."

According to Oremland, these results mean that this bacterium could be used to keep methyl bromide from escaping from the soil during field fumigations.

"Our tests showed that adding cells grown from methyl bromide or glucose to soil dramatically increased the rate at which methyl bromide dissipates," he said.

Fumigation levels of methyl bromide were consumed within 1 day in soils where methyl bromide-grown cells had been added and within 2 days for soil that had been treated with glucose-grown cells. It took 7 days for methyl bromide to disappear from unamended live soil.

"We also found that adding low levels of methyl iodide to the soil increased the dissipation rate of methyl bromide. Apparently this treatment increased the numbers of bacteria like IMB–1, thereby decreasing the time that methyl bromide stayed in the soil," Oremland said. Incorporating trimethylamine, a methylated amine, in the soil seemed to speed up this activity.

Oremland and colleagues plan field tests to see if these measures will eliminate methyl bromide emissions from the soil and still maintain the efficacy of the chemical as an agricultural fumigant.

At least one industry group thinks the new strain of bacteria might drastically cut the amount of methyl bromide that escapes into the air.

"We signed a cooperative research and development agreement with the U.S. Geological Survey to further test this strain of bacterium," said Tom Duafala. He is director of research and development of TriCal, a company located in Hollister, California, that specializes in custom application of soil fumigants.

Duafala said his company will provide experimental plots to test the following treatments or protocols:

- Lower the amount of chloropicrin from 33 percent to 2 percent, which would mean that chloropicrin would serve as a warning agent rather than as a fumigant.

- Add trimethylamine or a similar bacterial substrate like formate, ammonium formate, methanol, or methylated amines to the soil.

- Amend the soil with traces of methyl iodide and/or trimethylamine prior to fumigation with methyl bromide.

- Innoculate the soil with cultures of the new strain of bacterium before applying methyl bromide.

- Use a combination of the above measures.

"We hope to find a protocol or treatment that will provide a level of pest control conducive to the yield quantity and quality that growers normally get, while eliminating, or at least significantly decreasing, methyl bromide emissions," Duafala said. "Dr. Oremland and colleagues at the U.S. Geological Survey are working on ways to mass produce the bacteria for soil application. This project looks very promising."

Phyto-Oils Control Insects in Stored Products and Cut Flowers

Methyl bromide and phosphine are the most widely used fumigants for controlling pests in grain and dry, stored food products and quarantine insects in cut flowers for export. Some stored-product insects have reportedly developed resistance to phosphine. Phosphine also can cause corrosive damage if buildings and equipment are not built with noncorrosive materials. With the proposed phaseout of methyl bromide in 2001, growers are searching for alternatives.

Many aromatic plants, spices, and herbs can synthesize chemicals, such as essential oils, that kill or repel many insects but don't harm

mammals. The volatility and insecticidal efficiency of the oils make them good prospective fumigants. So scientists are studying essential oils as alternative natural fumigants to methyl bromide and phosphine.

Eli Shaaya, a professor with the Department of Stored Products, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel, is a visiting scientist at USDA's Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida. He and his colleagues conducted laboratory studies to evaluate the potential of these compounds.

"We used the most common stored-product insects—rice weevil (*Sitophilus oryzae*), lesser grain borer (*Rhyzopertha dominica*), sawtoothed grain beetle *(Oryzaephilus surinamensis)*, and red flour beetle *(Tribolium castaneum)*—as test insects. We also used two quarantine insects that attack cut flowers for export, the whitefly *(Bemisia tabaci)* on dianthus and thrips *(Frankliniella occidentalis)* on roses," says Shaaya.

The essential oils used in the tests were obtained from fresh plants, herbs, and spices, using steam distillation. Three types of tests were performed to evaluate the biological activity of oils. The first test was in space fumigation in chambers of 3.4 liters. Second, the highly active oils were tested in 600-milliliter fumigation chambers filled to 20 percent or 70 percent by volume with wheat. Finally, pilot tests were carried out in simulation columns 10 centimeters in diameter × 120 centimeters in height, filled to 70-percent volume with wheat.

Shaaya and his colleagues found that the oils under the code name ZP51 and SEM76 were the most effective against stored-product insects. In space fumigation, a concentration of only 1.4 to 4.5 g/m^3 of air was enough to obtain 90 percent adult mortality of all the insect species tested with a 1-day exposure time. For most of the other oils tested (about 50), a concentration of over 15 g/m^3 was needed to obtain 50-percent kill of *S. oryzae* and *T. castaneum*.

"In the second laboratory test, we evaluated the effectiveness of ZP51 against *S. oryzae* and *T. castaneum* in 600-milliliter chambers filled to either 20-percent or 70-percent volume with wheat," says Shaaya. "With 20-percent fill, concentrations of 3 and 10 g/m^3 of ZP51, and 1-day exposure were enough to cause 100-percent mortality of *S. oryzae* and *T. castaneum,* respectively."

"We found that with 70-percent fill, a concentration of 30 and 20 g/m^3 and exposure times of 2 and 3 days, respectively, were required to obtain 100-percent mortality for *S. oryzae*. A concentration of 40 g/m^3 and an exposure time of 4 days were needed to obtain 100-percent mortality of *T. castaneum*," says Shaaya.

In the final test, the scientists found that in columns filled 70 percent with wheat, a concentration of 50 g/m^3 and 5 days exposure were needed for 100-percent control of *S. oryzae* and *T. castaneum*. As for *R. dominica* and *O. surinamensis*, 7 days were needed for 100-percent mortality. And, if the concentration was increased to 70 g/m^3 with 4 days exposure time, 85–100 percent mortality of all insect species studied could be achieved.

"We demonstrated that the concentration of essential oils decreased gradually and only negligible amounts were found 3 months after treatment," Shaaya notes.

"A number of essential oils were also found active against cut-flower insects. Against the whitefly, a concentration of 10 g/m^3 and exposure of 2 hours were enough to obtain 100-percent mortality. In the case of thrips, a concentration of 20 g/m^3 and exposure time of 4 hours were needed to obtain 100-percent mortality. No phytotoxicity was observed 7 days after fumigation.

"Based on our studies, these two essential oils, ZP51 and SEM76, show great potential as fumigant alternatives to methyl bromide and phosphine. A concentration as low as 50 grams of oil/m^3 is enough to obtain effective control of stored-product insects, compared with the recommended concentration of 30–50 g/m^3 methyl bromide. In the case of cut flowers, a number of potential oils were also identified," says Shaaya.

Shaaya presented his research results at the Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions held in San Diego, November 3–5, 1997.

Reviving Propargyl Bromide, A Chemical From the Past

As the January 1, 2001, deadline for the ban on methyl bromide looms nearer, scientists from around the world intensify their search for viable alternatives.

Scott R. Yates is reviving interest in propargyl bromide, a chemical used with chloropicrin and methyl bromide in Trizone, a fumigant developed in the 1960s. Yates is a soil scientist with the Agricultural Research Service's U.S. Salinity Laboratory in Riverside, California. He reported this research at the Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions held in San Diego, November 3–5, 1997.

"The increasing popularity of methyl bromide back in the '60s is one reason that propargyl bromide was never commercialized," Yates reported. "Since the compound is not currently registered by the Environmental Protection Agency as a pesticide, its environmental behavior is essentially unknown."

In tests, Yates and colleagues at Riverside found that under typical agricultural conditions, this chemical appears to pose no serious environmental risk. "It degrades quickly in the soil. This would help limit the amount of the chemical that reaches groundwater or escapes into the atmosphere," he said.

Since virtually no information exists on propargyl bromide, Yates concentrated research efforts on basic parameters such as solubility, saturated vapor density, and the ease with which the chemical moves through the soil and escapes into the atmosphere. This knowledge is critical before a decision can be made on whether the chemical should be used as a soil fumigant, Yates said. The data are also needed to design methods that minimize application rates and optimize control of target organisms.

Yates compared the solubility of propargyl bromide (3BP) with that of methyl bromide and 1,3–dichloropropene (1,3–D), a potential methyl bromide alternative. Solubility determines how quickly a chemical is transported with water moving through porous substances.

"At low temperatures, the solubility of propargyl bromide increases rapidly as temperatures rise, and then levels off. We found that this chemical dissolves into water at a rate comparable to methyl bromide," Yates said. "And under high leaching conditions, more propargyl will move with water than 1,3–D, since 1,3–D is less soluble."

According to Yates, since it is important that a chemical move rapidly through the soil, all agricultural fumigants generally have high saturated vapor density. The saturated vapor density gives an indication of the tendency of a chemical to prefer the vapor phase.

In soil tests, 3BP degraded at a rate similar to 1,3–D, but much faster than methyl bromide. In theory, Yates said, that part of a chemical applied to the soil which doesn't degrade will eventually enter groundwater or the atmosphere. Since 3BP degrades rapidly in soil, it will have lower volatilization and is less likely to leach into groundwater than methyl bromide. "Because of its easy movement through and low retention rate in the soil, 3BP should provide a fairly uniform fumigation," said Yates. "Unless the soil is covered with a barrier like an impermeable plastic film, most agricultural fumigants will have a high rate of emission into the air. In fact, very high emission rates are reported for both methyl bromide and 1,3–D in field and lab tests.

"On one hand, high emission rates would reduce phytotoxicity and possible groundwater contamination. But on the other hand, air emissions could produce undesirable health effects on people living or working near treated fields. Since propargyl bromide is highly degradable, little would reach the atmosphere or groundwater, compared to methyl bromide," he reported.

Propargyl bromide has characteristics which suggest that it would be environmentally safe and, therefore, warrants consideration as a methyl bromide alternative, Yates said. "However, further research is needed to determine its effectiveness in controlling pests and to further study its fate and transport behavior under typical farming conditions."

Technical Reports

Grapefruit and Mexican Fruit Fly Tolerance to Refrigerated Controlled Atmosphere Storage

Principal Investigators: Krista C. Shellie, Research Plant Physiologist, and Robert L. Mangan, Research Entomologist, Crop Quality and Fruit Insects Research Unit, USDA–ARS, Weslaco, TX

The Mexican fruit fly, *Anastrepha ludens* (Loew), is undesirable in citrus production regions. Citrus fruit harvested from regions infested with fruit fly could contain unhatched eggs or larvae of various ages, depending upon the elapsed time between fly ovipositing and fruit harvest. Because infested fruit cannot be easily eliminated by external inspection, regulatory agencies in many countries have established phytosanitary quarantine protocols. Quarantine protocols include preharvest techniques, such as sterile fly release, non-host status, and pest-free growing periods, as well as postharvest commodity treatments, such as methyl bromide fumigation and cold storage. "All postharvest problems pale in comparison to . . . quarantines placed on fruit shipments due to an outbreak of exotic fruit flies" (Citrograph, 1992).

Among permitted treatments, methyl bromide is the only available fumigant, yet it can cause losses of fruit ranging up to 60 percent of loads treated at destination. If the citrus industry were forced to fumigate with methyl bromide before loading and shipment, losses in exports would be even higher, making methyl bromide fumigation an economically unacceptable treatment (Citrograph, 1992). Storage at 33 to 35 °F for 18 to 22 days is approved by the Animal and Plant Health Inspection Service of the United States Department of Agriculture as a quarantine treatment against Mexican fruit fly for citrus imported into the United States from Mexico or Central America. However, this treatment is not used commercially because of problems with fruit developing chilling injury. The objective of this research was to assess whether storage under a nonchilling temperature in an atmosphere with modified levels of oxygen or carbon dioxide could provide quarantine security against Mexican fruit fly without damaging fruit quality.

Tolerance of 'Rio Red' grapefruit, *Citrus paradisi* Macf., to storage at 50 °F in a hypoxic or hypercarbic atmosphere was evaluated in a series of small experiments. Grapefruit quality was evaluated after 14 or 21 days of storage in air, ultra low oxygen (0.05, 0.10, or 0.15 percent), or high carbon dioxide (20, 40, or 60 percent). Results from these experiments demonstrated that storage in either 40 or 60 percent carbon dioxide (balance air) caused a breakdown of flavedo tissue, and that fruit tolerated storage for up to 21 days in ultra low levels of oxygen (0.05, 0.10, or 0.15 percent) or 20 percent carbon dioxide (balance air). Grapefruit stored in 0.05 percent oxygen were rated for flavor as acceptable yet inferior to fruit stored in air or in 0.10 percent oxygen. Grapefruit stored in ultra low levels of oxygen had a lower incidence of decay, a higher amount of titratable acid, and a lower ratio of soluble solids to titratable acid than grapefruit stored in air.

The mortality of Mexican fruit fly larvae after storage in a refrigerated controlled atmosphere was also evaluated in a series of small experiments. The mortality of third instar larvae that were artificially infested into grapefruit and then stored for 14 or 21 days in air, 0.05 percent oxygen, 0.10 percent oxygen or 20 percent carbon dioxide (balance air) was found to be highest after storage in 0.05 percent oxygen. Six life stages of Mexican fruit fly were stored for 21 days on laboratory diet at 50 °F in 0.05 percent oxygen or in air. The number of surviving individuals were counted after removal from cold storage and holding under optimum rearing conditions. Late third instar larvae and eggs were found to be the life stages most likely to survive cold storage in 0.05 percent oxygen.

Responses observed in this study for grapefruit were similar to that reported by other researchers. Ke and Kader (1990) found that 'Valencia' orange (*citrus sinensis* (L.) Osbeck) tolerated exposure to 0.02 percent oxygen at 50 °F for up to 20 days without detrimental effects on external and internal appearance, but developed skin browning and poor external appearance after storage in 60 percent carbon dioxide. They also found that 'Valencia' oranges developed an acceptable, yet detectable off-flavor during storage in ultra low oxygen and they associated this off-flavor with an increase in

tissue concentration of ethanol and acetaldehyde. Davis et al. (1973) suggested that development of off-flavor under anaerobic conditions may also be attributed to a shift in equilibrium toward reduced forms of flavor compounds. Intolerance of grapefruit to storage in elevated levels of carbon dioxide has been reported by Stahl and Cain (1937) and Scholz, et al. (1960). Grapefruit from Florida (Stahl and Cain, 1937; Chace, 1969), and Texas (Scholz et al.,1960) have been shown to tolerate refrigerated storage in a low oxygen atmosphere for up to 4 weeks.

Low oxygen was also found to be more efficacious than elevated carbon dioxide for killing Mexican fruit fly larvae during heating in forced-air (Shellie et al., 1997). Grapefruit heated in 1 percent oxygen required 30 percent less exposure time than grapefruit heated in air to obtain 100 percent larval mortality. Results from these experiments suggest that storage in ultra low levels of oxygen at specific temperatures has potential for disinfesting grapefruit of the Mexican fruit fly.

Although quarantine uses of methyl bromide amount to only 5 to 8 percent of total U.S. consumption, this application will be especially difficult to replace. There are few candidate fumigants that have the necessary toxicity to the target pest, volatility to be useful in the required range of temperatures for treatment and acceptable phytotoxicity effects. The U.S. General Accounting Office reported in 1994 that $431,510,000 in U.S. exports were treated with methyl bromide as a condition of entry to receiving countries. Moreover, methyl bromide is currently the only emergency fumigant available to disinfest commodities from growing areas quarantined as a result of the invasion of exotic pests, such as the Mexican fruit fly. The loss of methyl bromide in 2001 will have significant negative impact on U.S. domestic and international trade. The research presented herein demonstrates the importance the Agricultural Research Service places on developing alternative commodity treatments to ensure a highly competitive, economically viable U.S. agricultural production system.

Botanical Extracts Reduce Populations of Soil Pathogens

Principal Investigators: James C. Locke and John H. Bowers, Research Plant Pathologists, Floral and Nursery Plants Research Unit, U.S. National Arboretum, USDA–ARS, Beltsville, MD 20705

There are a number of soilborne fungal pathogens that are of concern in the production of ornamental crops. To combat these pathogens, methyl bromide has been used to fumigate soil prior to planting many of these crops. One such example is Fusarium wilt of chrysanthemum, a widespread and destructive disease of this major horticultural crop. Symptoms of this disease may not become evident until near the time of flower production, although infection takes place early in the crop cycle.

The overall goal of this research project is to develop and evaluate new or existing, biologically based alternative control methods that can be integrated into cropping systems which currently use methyl bromide. Environmentally safe alternatives such as natural plant products, biological control agents, and cultural methods are being investigated. Major soilborne pathogens, *Fusarium, Rhizoctonia*, *Verticillium*, and *Phytophthora* are being used in bioassays designed to determine impact on soil populations and disease reduction in selected crops. This report focuses on the effect of several formulated plant extracts in reducing soil populations of *Fusarium oxysporum* f. sp. *chrysanthemi* (F.o.c.).

Artificially infested soil samples were treated with several rates of the botanical materials, incubated under standardized conditions, and assayed periodically to determine survival of the pathogen. The materials evaluated included the following formulations: 70 percent clove oil, 90 percent neem oil, pepper extract plus oil of mustard (4.94 percent capsaicin, 4.43 percent allyl isothiocyanate), cassia tree extract (Abion M), and the standard soil-drench fungicide Banrot 40W. *Fusarium* population determinations were made at 0, 1, 3, 7, 14, and 21 days after soil treatment. The natural product formulations were applied as 1, 5, and 10 percent aqueous emulsions at 5 ml per 150 cubic centimeters of soil.

Neither Banrot nor 1 percent applications of any of the botanical extracts reduced the population of F.o.c. compared to the untreated control. However, the 5 and 10 percent applications of the botani-

cal extracts resulted in significant differences in the surviving F.o.c. populations at each assay date. Treatment with the neem oil material actually resulted in increased F.o.c. populations.

Soil populations of F.o.c. were lowest after 3 to 7 days of incubation with the pepper extract. In addition, populations of other soil microbes were also reduced to the greatest degree when treated with the pepper extract. The F.o.c. populations did rebound over time, showing a steady increase beginning 7 days after treatment. This increase could be the result of either breakdown of the pepper extract in soil or the lack of microbial competition which allows any surviving F.o.c. propagules to rapidly recolonize the treated soil. This may also occur with the clove oil and cassia extract but to a lesser extent. Further research will address these population dynamics questions.

The ability of these natural products to reduce soil populations of these pathogens coupled with their environmentally friendly composition makes these materials attractive candidates for use in biologically based management strategies. In the highly intense management of high value ornamental crops, these materials may be part of the answer to the quest for an alternative to methyl bromide fumigation. However, to fully develop an alternative cultural practice, these materials may need to be integrated with other practices such as the use of beneficial or biocontrol agents.

Additional research needs to be completed to scale-up these findings to actual production systems. Application rates and methods, pre-plant incubation periods, tolerance of various crop plants, and impact on soil microbial systems are being addressed. Whether this technology could be adapted for use with agronomic crops remains to be determined.